Kidney International, Vol. 61, Supplement 80 (2002), pp. S143–S148

Control of uremic bone disease: Role of vitamin D analogs

EDUARDO SLATOPOLSKY, ADRIANA DUSSO, and ALEX J. BROWN

Washington University School of Medicine, Renal Division, St. Louis, Missouri, USA

Secondary hyperparathyroidism (SH) is a universal complication of chronic renal failure. In early renal failure, alteration in vitamin D metabolism plays a key role in the development of SH [1–4]. Low levels of $1,25(OH)_2D_3$ and decreased repression of the PTH gene transcription may allow a greater synthesis and secretion of PTH. As renal disease progresses, the number of vitamin D receptors (VDR) in the parathyroid glands (PTG) decrease [5–8]; thus, the PTG becomes resistant to the action of $1,25(OH)_2D_3$. In addition, "uremic toxins" may further decrease the suppressive effect of $1,25(OH)_2D_3$ [9]. Concomitantly with the above-described alterations, hyperplasia of the PTG develops and a decreased number of calcium receptors (CaR) [10, 11] further increases the resistance of the PTG to serum ICa. Thus, higher serum calcium is necessary to suppress SH. Recently, investigators examined the clonality of hyperplastic tumors using X-chromosome inactivation analysis [12]. In about two thirds of uremic patients with refractory S.H. harbored at least one monoclonal parathyroid tumor. Phosphate independent of serum Ca and $1,25(OH)_2D_3$ increases PTH synthesis and secretion by a post-transcriptional mechanism [13–19]. Dietary phosphate also regulates parathyroid growth [20]. Low phosphate diet increases p21, a repressor of the cell cycle and inhibitor of parathyroid gland hyperplasia, while high phosphate enhances transforming growth factor alpha (TGFα) and the epidermal growth factor receptor (EGFR) known to play an important role on cell proliferation.

The vitamin D hormone, $1,25(OH)_2D_3$ (calcitriol), the most active metabolite of vitamin D, controls parathyroid gland growth and suppresses the synthesis and secretion of parathyroid hormone. Because of its effects on PTH suppression, calcitriol has been successfully used in the treatment of secondary hyperparathyroidism that almost always accompanies chronic renal failure [21, 22]. The efficacy of intravenous calcitriol in suppressing PTH in patients with secondary hyperparathyroidism is well established [23, 24]. However, because of its potent effects on intestinal calcium and phosphate absorption and bone calcium and phosphate mobilization, calcitriol can induce hypercalcemia and hyperphosphatemia, often precluding its use at therapeutic doses. Therefore, an analog of calcitriol that retains the therapeutic effects but has minor effects on calcium and phosphate metabolism would be an ideal tool for the treatment of secondary hyperparathyroidism.

Key words: vitamin D, hyperparathyroidism, uremia, hypercalcemia, hyperphosphatemia.

VITAMIN D ANALOGS

The biological actions of calcitriol are mediated by a nuclear VDR. At present, there is evidence for only a single form of the VDR. Thus, the same VDR mediates both the calcemic actions and the non-classical potentially therapeutic actions of calcitriol. The novel aspect of recently developed analogs is their differential actions, compared to calcitriol in vivo. In fact, as these analogs have a relatively high affinity for the vitamin D receptor, usually within one order of magnitude, it is not unexpected that they are able to mimic many of the actions of calcitriol in vivo. The unique feature of therapeutically useful analogs is their ability to efficiently support some but not all calcitriol associated activities. The potential mechanisms through which this selectivity could be achieved are summarized in Figure 1. Most commonly, the analogs display decreased potency in enhancing intestinal absorption or bone mobilization of calcium and phosphate. The selectivity is not always cell or tissue specific but can be gene or process specific within the same tissue.

The structure-activity relationship for ligand-mediated transcriptional regulation has been studied in detail [25]. The A-ring structure is most crucial, especially the hydroxyl groups, for binding to the VDR. Modification of the D-ring or side chain does not greatly affect VDR binding, but can influence biological potency by altering the pharmacokinetics or catabolism. Analogs can also produce distinct conformational changes in the VDR that may produce gene-specific actions. A combination of structural modifications can produce analogs with diverse biological profiles.

Recruitment of coactivators or co-repressors also can play an important role on vitamin D analogs transcrip-

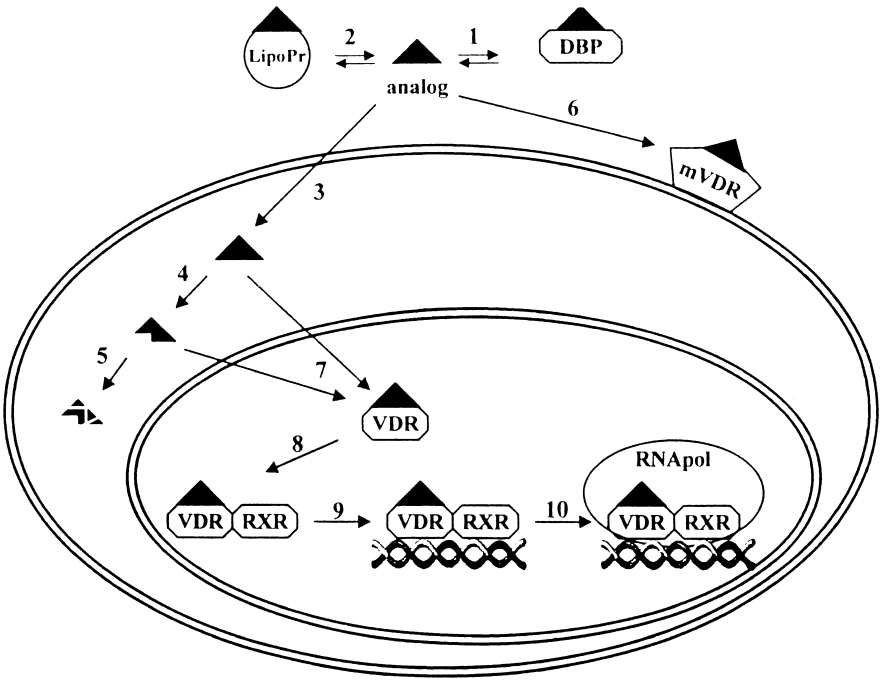

Fig. 1. Potential sites of differential actions of $1,25(OH)_2D_3$ and its analogs. The possible steps in the vitamin D activation pathways at which differences in vitamin D analog action could lead to selective activities in vivo are shown. The steps diagrammed include: *1*) DBP affinity, *2*) interaction with other serum proteins including lipoproteins, *3*) cellular uptake, *4*) conversing to active metabolites, *5*) catabolic inactivation, *6*) activation of the nongenomic pathway through a membrane vitamin D receptor (mVDR), *7*) interaction with the nuclear vitamin D receptor (VDR), *8*) formation of the VDR-RXR complex, *9*) binding to the activated complex to DNA, and *10*) formation of the preinitiating complex RNA polymerase II (RNApol) (reproduced from [40]; used with permission).

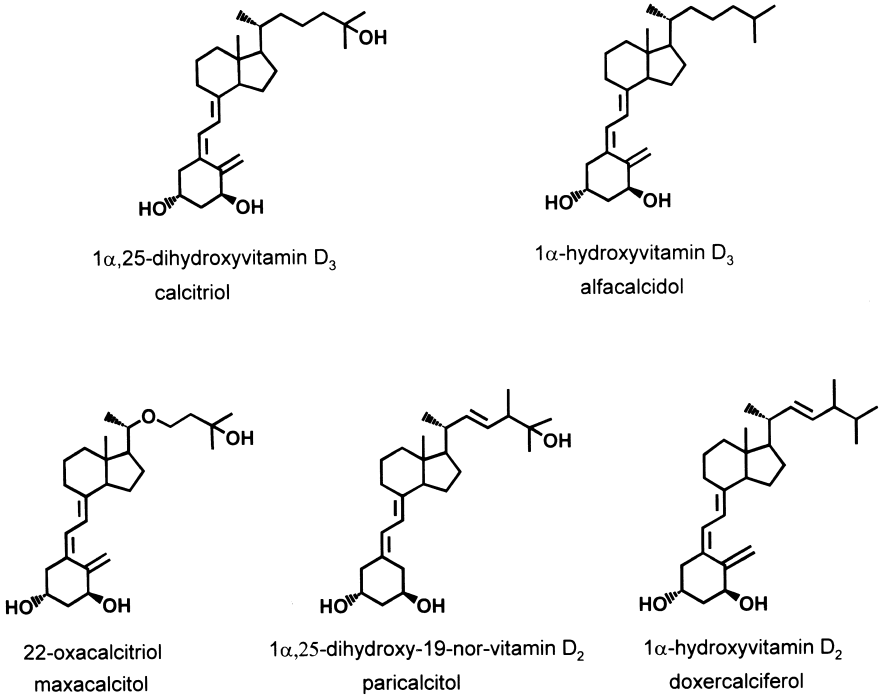

Fig. 2. Chemical structure of calcitriol and several vitamin D analogs.

tion-induced biological actions. Work from Takeyema et al [26] demonstrated that calcitriol can recruit binding of several coactivators to the VDR that may enhance the activation of transcription, whereas 22 oxa-calcitriol recruits only a subset of these, which could potentially produce biological effects distinct from those of calcitriol.

22-OXACALCTRIOL (OCT)

OCT differs from calcitriol only by a substitution of an oxygen in place of carbon 22 in the side chain (Fig. 2). The affinity of 22-oxacalcitriol for the VDR is about 8 times lower than that calcitriol, consistent with its lower activity in suppressing PTH. Studies in animals demon-

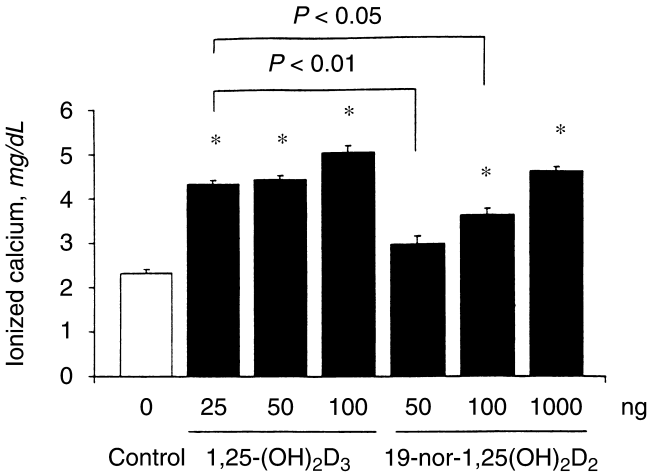

Fig. 3. Effects of $1,25(OH)_2D_3$ and 19-nor $1,25(OH)_2D_2$ on plasma ionized calcium levels in parathyroidectomized rats fed a calcium-deficient diet. Rats were given daily injections of vehicle (white bar), $1,25(OH)_2D_3$, or 19-nor-$1,25(OH)_2D_2$ for 9 days.

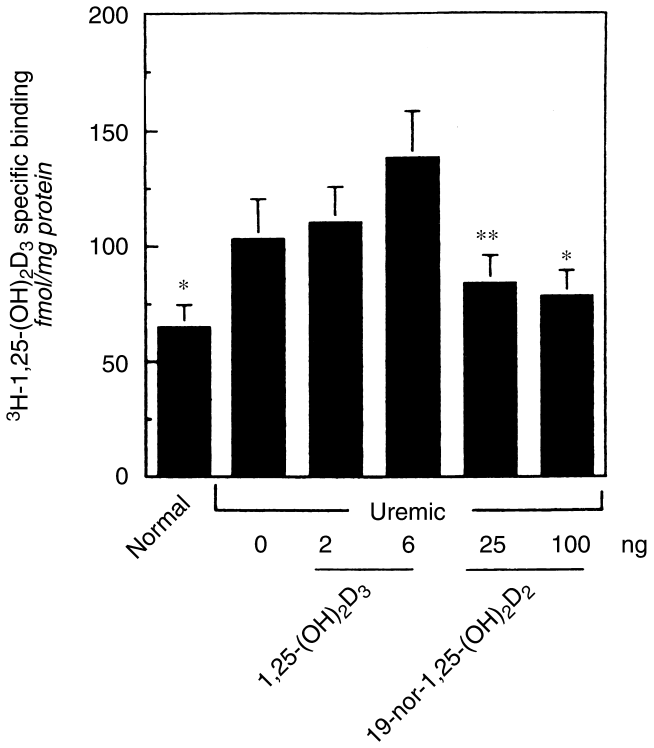

Fig. 4. Effects of $1,25(OH)_2D_3$ and 19-nor $1,25(OH)_2D_2$ on intestinal $1,25(OH)_2D_3$– VDR binding in uremic rats. Rats were treated with vehicle, $1,25(OH)_2D_3$ (2 or 6 ng), or 19-nor-$1,25(OH)_2D_2$ (25 ng or 100 ng) three times a week for 8 weeks. All data are mean ± SEM. N = 11 to 15 rats per group. Asterisk and double asterisk indicate $P < 0.01$ an $P < 0.05$ versus uremic + $1,25(OH)_2D_3$–6 ng, respectively (reproduced from [36]; used with permission).

strated that OCT is rapidly cleared from the circulation; the short half life may be secondary to diminished affinity for DBP that is approximately 400–500 times less than of calcitriol [27–30]. The low calcemic and phosphatemic effect of OCT may be secondary to low affinity for DBP. Currently the mechanism for the differences in the duration of the effects in the parathyroid glands versus the intestine and bone are not completely understood, but the findings indicated that stimulation of intestinal calcium absorption and bone resorption are short-lived responses that require continuous exposure to vitamin D compounds. On the other hand, even a short exposure of the parathyroid gland to OCT leads to a prolonged suppression of PTH. The mechanism of PTH suppression by OCT was similar to that of calcitriol in that the analog decreased PTH mRNA, suggesting that it was also acting at the level of gene transcription [31]. Studies in animal models with experimental renal failure demonstrated that OCT was able to suppress PTH over a wide dose range with no change in serum calcium. In contrast, doses of calcitriol just above those that suppress PTH produced a significant increase in serum calcium. The effect of OCT on renal osteodystrophy was examined by Monier-Faugere et al [32] in dogs made uremic by subtotal nephrectomy. OCT significantly decreased PTH levels. The analog reversed abnormalities in bone formation, including woven osteoid and fibrosis. However, no change in the rate of bone turnover was observed. While hypercalcemic episodes occurred, OCT did not induce low turnover bone disease. OCT significantly reduced bone marrow fibrosis and decreased markers of bone turnover in patients with end stage renal failure [33].

19-NOR $1,25(OH)_2D_2$ (19-NOR)

This vitamin analog lacks the exocyclic carbon 19 and has a vitamin D_2 side chain (double bone in carbon 22 and extra carbon in 28 position) (Fig. 2). We demonstrated that 19-nor $1,25(OH)_2D_2$ suppress parathyroid hormone secretion in primary cultures of bovine parathyroid cells as potently as calcitriol [34]. In addition, this compound can suppress pre-pro PTH messenger RNA and PTH secretion without inducing hypercalcemia or hyperphosphatemia.

Daily administration of 19-nor $1,25(OH)_2D_2$ to parathyroidectomized rats fed either a calcium or a phosphorus-deficient diet for 9 days produced smaller increases in plasma calcium and phosphate than calcitriol. Dose-response studies demonstrated that 19-nor $1,25(OH)_2D_2$ is approximately 10 times less active than calcitriol in mobilizing calcium and phosphate from bone (Fig. 3) [35]. Moreover, in contrast to calcitriol, which up regulates the VDR in the intestine, 19-nor-$1,25(OH)_2D_2$ has the opposite effect [36] (Fig. 4).

The efficacy of 19-nor $1,25(OH)_2D_2$ in renal failure patients was demonstrated in a recent study [37] in 78 patients. Placebo was given to approximately one third

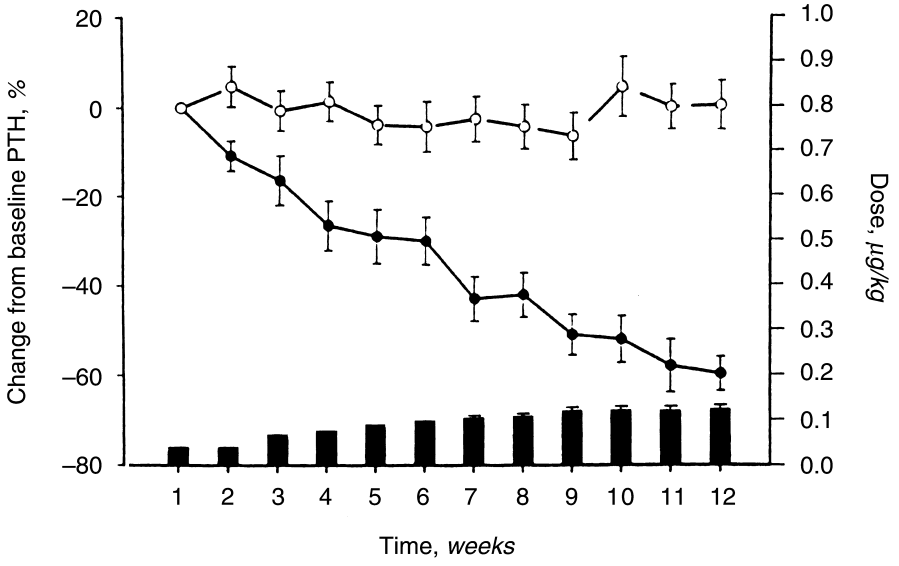

Fig. 5. Changes in the levels of intact PTH expressed as a percentage of change from baseline values during the study period in placebo-treated (open circle) and paricalcitriol-treated (closed circle) groups. The bars depict the doses of paricalcitriol that increase according to protocol. (Reproduced from [37]; used with permission).

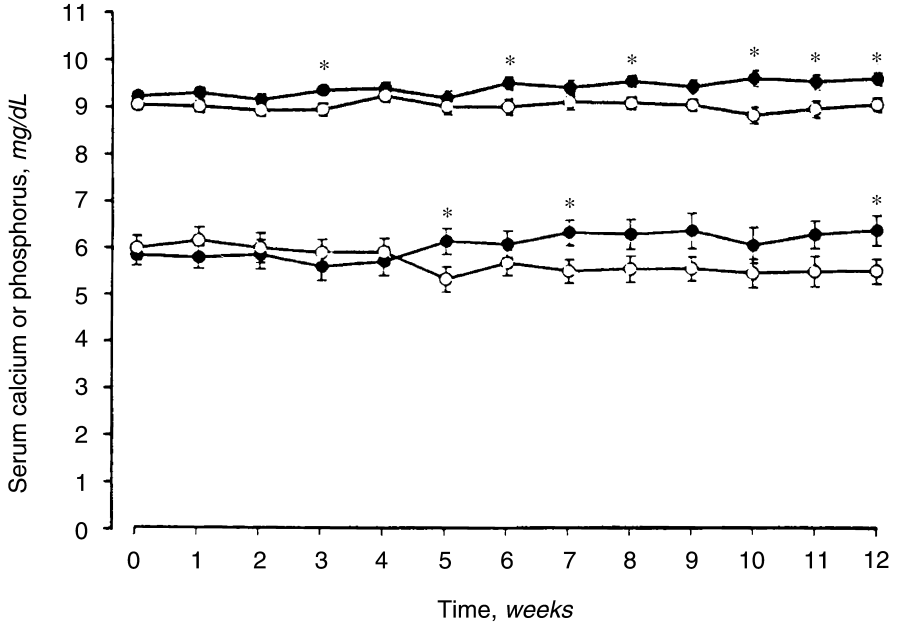

Fig. 6. The values for normalized serum calcium (upper lines) and serum phosphorus (lower lines) during the 12 weeks of study in placebo (open circle) and paricalcitriol (closed circle) groups. $*P < 0.05$. (Reproduced from [37]; used with permission).

of the patients and 19-nor $1,25(OH)_2D_2$ was administered to the other two-thirds The dose was initially 0.04 μg/kg and rose to an average of 0.12 μg/kg during the course of the 7 week study. Serum PTH levels dropped an average of approximately 60% (Fig. 5) with only a slight increase in serum calcium, from 9.24 ± 0.12 to 9.5 ± 0.15 (Fig. 6).

$1\alpha(OH)D_2$

This vitamin D analog is a pro-hormone and must be converted by the liver to $1,25(OH)_2D_2$ before it becomes an active compound (Fig. 2). The basis for the low calcemic activity is much less understood. Early studies with $1\alpha(OH)D_2$ show that it is less toxic than $1\alpha(OH)D_3$ when the compounds were administered chronically [38]. Paradoxically, the stimulation of calcium transport and bone mobilization by $1\alpha(OH)D_2$ and $1\alpha(OH)D_3$ were not different [38]. Recently Maung et al [39] reviewed their experience with $1\alpha(OH)D_2$. The investigators found, in patients with renal failure, that both oral and intravenous preparations of $1\alpha(OH)D_2$ were effective in controlling secondary hyperparathyroidism. They also noted smaller increments in serum calcium and phosphorus levels with intravenous administration compared with oral therapy. However, the prevalence of hypercalcemia and hyper-

phosphatemia still remains high with the IV therapy. Serum calcium increased above 10.5 mg/dl in 8.4% of patient and serum phosphorus above 6.8 mg/dl in 13.5% of patients.

CONCLUSION

Vitamin D analogs with improved specificity are now available for treatment of secondary hyperparathyroidism. Studies in uremic rats have demonstrated that OCT and 19-nor $1,25(OH)_2D_2$ have a wider therapeutic window than $1,25(OH)_2D_3$ due to their lower calcemic and phosphatemic effects on the intestine and bone. Studies in humans have shown their analogs to be less calcemic and phosphatemic than calcitriol but further studies will be necessary to confirmed their parathyroid selectivity in renal patients.

The mechanisms responsible for the lower calcemic and phosphatemic activities of these analogs vary. The reduced effect of OCT on intestine and bone is attributed to its altered pharmacokinetics and possibly to restricted coactivator recruitment by the OCT-VDR complex. The molecular basis for the lower calcemic and phosphatemic effect of 19-nor $1,25(OH)_2D_2$ and $1\alpha(OH)D_2$ is under investigation. A clearer understanding of how these analogs exert their selectivity may allow the design of the future analogs with a greater specificity for suppressing secondary hyperparathyroidism in renal failure.

ACKNOWLEDGMENTS

This work was supported in part by a grant provided by Abbott Pharmaceutical Company and Research in Renal Diseases, Washington University.

Reprint requests to Eduardo Slatopolsky, M.D., Washington University School of Medicine, Renal Division, 660 S. Euclid Avenue, Box 8126, St. Louis, MO 63110, USA.
E-mail: eslatopo@im.wustl.edu

REFERENCES

1. Chesney RW, Hamstra AJ, Mazess RB, *et al*: Circulating vitamin D metabolite concentrations in childhood renal disease. *Kidney Int* 21:65–72, 1997
2. Juttmann JR, Burman JC, Dekam E, *et al*: Serum concentrations of metabolites of vitamin D in patients with renal failure. *Clin Endocrinol* 14:225–232, 1981
3. Wilson L, Felsenfeld A, Drezner MD, Llach F: Altered divalent ion metabolism in early renal failure: Role of $1,25(OH)_2D_3$. *Kidney Int* 27:565–570, 1985
4. Martinez I, Saracho R, Montenegro J, Llach F: A deficit of calcitriol synthesis may not be the initial factor in the pathogenesis of secondary hyperparathyroidism. *Nephrol Dial Transplant* 11:22–28, 1996
5. Korkor AB: Reduced binding of [^{3}H]1,25-dihhydroximtmian D_3 in the parathyroid glands of patients with renal failure. *N Engl J Med* 316:1573–1577, 1987
6. Merke J, Hugel U, Zlotkowski A Szabo A, Bommer J, Mall G, Ritz E: Diminished parathyroid $1,25(OH)_2D_3$ receptors in experimental uremia. *Kidney Int* 32:3350–3353, 1987
7. Brown AJ, Dusso A, Lopez-Hilker S, *et al*: $1,25(OH)_2D_3$ receptors are decreased in parathyroid glands from chronically uremic dogs. *Kidney Int* 35:19–23, 1989
8. Fukuda N, Tanaka H, Tominaga Y, *et al*: Decreased 1,25-Dihydroxyvitamin D_3, receptor density is associated with a more severe form of parathyroid hyperplasia in chronic uremic patients. *J Clin Invest* 92:1436–1443, 1993
9. Patel SR, Keh W, VanHolder R, *et al*: Inhibition of calcitriol receptor biding to vitamin D response element by uremic toxins. *J Clin Invest* 96:50–59, 1995
10. Kifor O, Moore FD, Wang P, *et al*: Herbert SC Brown EM. Reduced immunostaining for the extra-cellular Ca sensing receptor in primary and uremic secondary hyperparathyroidism. *J Clin Endocrinol Metab* 81:1598–1606, 1996
11. Gogusev JT, Duchambon P, Hory B, *et al*: Depressed expression of calcium receptor in parathyroid gland tissue of patients with hyperparathyroidism. *Kidney Int* 51:328–336, 1997
12. Arnold A, Brown MF, Ureña P, *et al*: Monoclonality of parathyroid tumors in chronic renal failure and in primary parathyroid hyperplasia. *J Clin Invest* 95:2047–2053, 1995
13. Lopez-Hilker S, Dusso AS, Rapp NS, Martin KJ, Slatopolsky E: Phosphorus restriction reverses hyperparathyroidism in uremia independent of changes in calcium and calcitriol. *Am J Physiol* 259:F432–F437, 1990
14. Slatopolsky E, Finch J, Denda M, *et al*: Phosphorus restriction prevents parathyroid gland growth-high phosphorus directly stimulates PTH secretion in vitro. *J Clin Invest* 97:2534–2540, 1996
15. Almaden Y, Canalejo A, Hernandez A, *et al*: Direct effect of phosphorus on PTH secretion from whole rat parathyroid glands in vitro. *J Bone Miner Res* 11:970–976, 1996
16. Moallem E, Kilav R, Silver J, Naveh-Many T: RNA-protein binding and post-transcriptional regulation of parathyroid hormone gene expression by calcium and phosphate. *J Biol Chem* 273:5253–5259, 1998
17. Naveh-Many T, Rahamimov R Livni N, Silver J: Parathyroid cell proliferation in normal and chronic renal failure in rats. The effects of calcium, phosphate, and vitamin D. *J Clin Invest* 96:1786–1793, 1995
18. Denda M, Finch J, Slatopolsky E: Phosphorus accelerated the development of parathyroid hyperplasia and secondary hyperparathyroidism in rats with renal failure. *Am J Kidney Dis* 28:596–602, 1996
19. Kilav R, Silver J, Naveh-Many T: A conserved cis-acting element in the parathyroid hormone 3′-untranslanted region is sufficient for regulation of RNA stability by calciun and phosphate. *J Biol Chem* 276:8727–8733, 2001
20. Dusso A, Pavlopoulos T, Naumovich L, *et al*: p21 and TGF alpha mediate dietary phosphate regulation of parathyroid cell growth. *Kidney Int* 59:855–865, 2001
21. Slatopolsky E, Weerts C, Thielan J, *et al*: Marked suppression of secondary hyperparathyroidism by intravenous administration of 1,25-dihydroxycholecalciferol in uremic patients. *J Clin Invest* 74:2136–2143, 1984
22. Delmez JA, Tindira C, Grooms P, *et al*: Parathyroid hormone suppression by intravenous $1,25(OH)_2D$: A role for increased sensitivity to calcium. *J Clin Invest* 83:1349–1355, 1989
23. Andress DL, Norris KC, Coburn JW, *et al*: Intravenous calcitriol in refractory osteitis fibrosa of chronic renal failure. *N Engl J Med* 321:274–279, 1989
24. Gallieni M, Brancaccio D, Padovese P, *et al*: Low-dose intravenous calcitriol treatment of secondary hyperparathyroidism in hemodialysis patients. *Kidney Int* 42:1191–1198, 1992
25. Bouillon R, Okamura WH, Norman AW: Structure-function relationship in the vitamin D endocrine system. *Endocrinol Rev* 16: 2001–2057, 1995
26. Takeyama K, Masuhiro Y, Fuse H, *et al*: Selective interaction of vitamin D receptor with transcriptional coactivators by a vitamin D analog. *Mol Cell Biol* 19:1049–1055, 1999
27. Dusso AS, Negrea L, Gunawardhana S, *et al*: On the mechanisms for the selective action of vitamin D analogs. *Endocrinology* 128: 1687–1692, 1991
28. Okano T, Tsugawa N, Masuda S, *et al*: Protein binding properties of 22-oxacalcitriol 1α,25-dihydroxyvitamin D_3, a synthetic ana-

logue of 1α,25-dihydroxyvitamin D_3. *J Nutr Sci Vitaminol* 35:529–533, 1989
29. Brown AJ, Finch J, Grieff M, *et al*: The mechanism for the disparate actions of calcitriol and 22-oxacalcitriol in the intestine. *Endocrinology* 133:1158–1164, 1993
30. Kobayashi T, Tsugawa N, Okano T, *et al*: The binding properties, with blood proteins and tissue distribution of 22-oxacalcitriol 1α,25-dihydroxyvitamin D_3, a noncalcemic analogue 1 α,25-dihydroxyvitamin D_3, in rats. *J Biochem* 115:373–380, 1994
31. Brown AJ, Ritter CR, Finch J, *et al*: The non-calcemic analog of vitamin D, 22-oxacalcitriol (OCT) suppresses parathyroid hormone synthesis and secretion. *J Clin Invest* 84:728–732, 1989
32. Morinier-Faugere MC, Geng Z, Friedler RM, *et al*: 22-Oxacalcitriol suppresses secondary hyperparathyroidism without inducing low bone turnover in dogs with renal failure. *Kidney Int* 55:821–832, 1999
33. Tsukamoto Y, Hanaoka M, Matsuo T, *et al*: Effect of 22-oxacalcitriol on bone histology of hemodialysed patients with severe secondary hyperparathyroidism. *Am J Kidney Dis* 35:458–464, 2000
34. Slatopolsky E, Finch J, Ritter C, *et al*: A new analog of calcitriol, 19-nor-D_2 suppresses parathyroid hormone secretion in uremic rats in the absence of hypercalcemia. *Am J Kidney Dis* 26:852–860, 1995
35. Finch JL, Brown AJ, Slatopolsky E: Differential effects of 1,25-dihydroxyvitmain D_3 and 19-nor 1,25 dihydroxyvitamin D_2 on calcium and phosphorus resorption in bone. *J Am Soc Nephrol* 10:980–985, 1999
36. Takahashi F, Finch J, Denda M, *et al*: A new analog of $1,25(OH)_2D_3$, 19-nor $1,25(OH)_2D_2$ suppresses serum PTH and parathyroid gland growth in uremic rats without elevation of intestinal vitamin D receptor content. *Am J Kidney Dis* 30:105–112, 1997
37. Martin KJ, Gonzalez EA, Gellens M, *et al*: 19-nor-1 alpha-25-dihydroxyvitamin D_2 (paricalcitol) safety and effectively reduces the levels of intact PTH in patients on hemodialysis. *J Am Soc Nephrol* 10:1427–1432, 1998
38. Sjoden G, Smith C, Lindgren U, Deluca HF: 1 alpha-hydroxyvitamin D_2 is less toxic then 1 alpha-hydroxyvitamin D_3 in the rat. *Proc. Soc. Exp.* 178:432–436, 1985
39. Maung HM, Elangovan L, Frazao J: Efficacy and side effects of intermittent intravenous and oral doxecalciferol 1 $\alpha(OH)D_2$ in dialysis patients with secondary hyperparathyroidism: A sequential comparison. *Am J Kidney Dis* 37:5372–5543, 2001
40. Brown AJ: Mechanisms for the selective actions of vitamin D analogs. *Curr Pharm Des* 6:701–716, 2000